

REVIEW

Rhythmic Processes in Lower Vertebrate Embryogenesis and Their Role for Developmental Control

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ABSTRACT—A great number of various rhythmic processes (biochemical, biophysical, cytological) during an animal embryogenesis is described up to now. Many of them are revealed in the course of cleavage. The duration of a single cycle of these processes is usually equal to the duration of a single cleavage division (τ_0).

The somitogenesis is demonstrated the feature of rhythmic process too. We studied thoroughly increase of somites in many fish species, particularly in slow-developing salmon fishes allowing most exact measurements. It was shown that the formation of somites, next one after another, occurred (at constant conditions) strictly rhythmically with the same interval (τ_s). The last is specific for each species. It turned out that in embryos of the same species at the resemble temperature interval τ_s was equal τ_0 . These data permit to suppose that both embryonic processes are governed by the same intrinsic rhythm, peculiar to all cells of the embryo. This general conclusion is sustained by the data showing that in some salmon hybrids when the reorganization of the rhythms occurred a similar shift in the frequency of the ones for both processes was observed. It is possible that the autonomous changes in the competence of amphibian ectoderm for induction are also governed by the same endogenous rhythm.

The endogenous rhythm described could be responsible for switching on/off various specific programmes of development, and also for the coordination of numerous developmental events of embryogenesis. It is supposed that various programmes switched on/off by the same rhythmic signal can be performed independently.

INTRODUCTION

In adult organism, temporal coordination of various aspects of life processes is usually accomplished by means of periodic external phenomena like day and night, seasons, etc. Do organisms have any internal time control mechanism during the embryogenesis?

This period is remarkable for relative concise and extraordinary eventfull processes of differentiation and morphogenesis. One is tempted to ask how the activity of enormous numbers of genes is organized, not forgetting that gene expression is only the first step in the multistage

process when the realization of genes in characters occurs.

It has been generally believed that the development of an organism is a chain of consecutive stages: "Development seems to occur in a stepwise fashion, the completion of each step setting the stage for the next" [1]. If this is the case, the duration of a process depends on environmental factors (temperature, oxygen, pH, etc.), that's say the time is derivative of those ones, and does not play the role of the *primary* factor of development.

The proponents of this point of view believe that the duration of different embryonic processes is usually not stirtcly constant exploring it by the imperfections of control of each process. This hypothesis provides the basis for an opinion that

the development of embryos from one batch is not simultaneous, as it is shown by non-simultaneous hatching of embryos.

According to Crisp [2] the duration of embryogenesis is one batch estimated at this stage can differ by 20%. But hatching depends on a set of environmental factors and can occur at different stages of development [3].

The point of view that embryogenesis is a process with relatively variable time limits is beginning to change actually. This is the consequence of more appropriate methods using allowing to estimate quantitative aspects of embryonic development and of a series of direct experiments which are studying the time factor in the process of development. Several rhythmic processes in embryos indicate the existence of some embryonic time control mechanism or "biological clock" [4].

Several of these rhythmic processes showing similarities in particular aspects are described below.

RHYTHMIC PROCESSES DURING CLEAVAGE

Cleavage is the earliest period of the embryogenesis, but several rhythmic processes, including cell divisions, are observed already at this stage. These cleavage cell divisions occur synchronously in 10–12 cycles [5–6] with approximately equal time interval between similar phases of mitosis. This interval was designated τ_0 [7].

As it has been recently shown, rhythmic changes also occur during this period in biochemical and biophysical parameters. The most interesting ones are periodic changes of rigidity in the superficial layer of the egg cytoplasm [8], flattening-rounding of the egg and surface contraction waves and other changes [9–11] (for review of these and other changes see [12–14]). These processes occur at time intervals, which are approximately equal in duration to the cell cycle duration in cleavage. They occur both in intact and enucleated eggs and in egg fragments not containing nucleus. Besides, it has been shown that the changes of an electric activity of embryonic membranes occur also with a period equal to the cell cycle [15–16].

THE TIME CONTROL IN EMBRYOS AND SINGLE EMBRYONIC CELLS AT STAGES FOLLOWING CLEAVAGE

How is the time controlled in embryo cells after cell divisions are desynchronized during blastulation? The following answer on this question is possible: since the cyclic changes of some indices remain after the suppression of cleavage [12–14] it could be supposed that the same occurs when the cell cycle reorganization and the loss of rhythm by cell divisions takes place in intact embryos, namely rhythm of another processes retain. Some data may be presented, which prove the existence of temporal control in the embryos and their cells following the cleavage.

It has been ascertained in series of experiments that the beginning of blastulation and gastrulation in different groups of animals does not depend on the nuclear-cytoplasm ratio or on the number of divisions, but on the duration of development [17–19]. It has been shown also that the time of realization of different processes in either anlagen of embryo programmed in the cells and the schedule of their development (with one moment or another) occurs independently from neighbouring anlagen. For example, at the fusing of starfish embryos at different stages of development, in spite of the formation of highly permeable contacts between them, every embryo starts the gastrulation by own schedule [20]. The attempts to change the time of beginning of gastrulation using premature processing of embryos with the mesoderm-inducing factors have been unsuccessful [21]. The latter authors think that each cell in the early embryo contains a copy of the schedule controlling the correct initiation of developmental events, whether or not that cell has been instructed to participate in each event. Grainger and Gurdon [22] have shown that the loss of ability of mesodermal induction in ectodermal cells does not depend on cellular interactions and cell divisions: isolated cells lost their competence simultaneously with intact embryos. Further it has been demonstrated [23] that in *Xenopus* the cultured ectoderm undergoes the set of successive changes: first it is competent to form mesoderm, then neural tissue and finally placodal tissue. The authors conclude

that the competence of ectoderm changes over developmental time, and that no tissues interactions are required for these changes in competence.

It seems that the time control mechanism keeps at later stages and has certain significance for morphological differentiation of cells already in the separate anlagen of organs. So, the ability of chick embryo limb bud cells to differentiate into distal or proximal region of the wing is dependent on the duration of a period which the cells spend in the growth zone [24].

It seems that switching on/off the synthesis of several specific enzymes and chemical substances is also controlled by an intracellular embryo clock. A tissue-specific enzyme acetylcholinesterase (ACh E) is found in muscle cells of *Ascidia* embryos [25]. If at the stage of 32 blastomeres ascidian eggs were treated with cytochalasin (which inhibits cytokinesis, but does not suppress nuclear divisions) or colchicine (which inhibits nuclear divisions) in these seemingly undeveloping embryos ACh E activity appeared at the same moment as in intact embryos [12, 25, 27]. Caplan [27] also pointed to the existence of a biological clock in the synthesis the specific proteoglycans in chondrocytes both in embryonic organs and isolated cells.

SOMITHOGENESIS AS A RHYTHMIC PROCESS

The axial zone in vertebrates is subject to segmentation the first step of which is separation of parachordal mesodermal layers in rostro-caudal direction and formation of somites. They are formed in pairs, by one on each side of chorda. Their total number ranges from several tens to several hundreds of pairs. As has been shown earlier, the formation of somites is a relatively rhythmic process [28-30].

We studied somitogenesis in several species of teleosts at different temperatures. Teleosts embryos represent a convenient model for live observations since they are transparent.

In the laboratory the fertilized eggs of different fish species placed into special apparatus for incubation of salmon (and also other) fishes. During

incubation normal conditions of spawn development (oxygen concentration, flowing water, constant temperature ($\pm 0.1^\circ\text{C}$) maintained carefully. Within the optimal temperatures (for salmon eggs $2-10^\circ\text{C}$) our incubators are a success to provide the survival of 95-98% embryos up to hatching [31, 33].

In the course of somitogenesis we took out the samples of eggs for counting of somite pairs from the same batch from time to time. For the sake of this the chorions were removed microsurgically in a twofold Holtfreter solution, the yolk was drawn off and embryos were carried by pipette on the preparation glass. Then we counted the number of somite pairs in every embryo under microscope (enhance $7\times$). The production of 5-10 living embryo preparations and the counting of somites in them usually took no more than 30-50 min. Numbers of somite pairs in each sample under investigation were averaged and plotted on the graphic of increase of somite pair sum in dependence on the incubation duration.

In a series of observations of embryos from one batch it was found that at constant temperature the total number of somites is a linear function of incubation time (Fig. 1; see also [3, 31-34]). This rule is retraced from the laying of first somite pair up to 35 pairs in perca [34], 57 pairs in pike [32], 60 pairs in Atlantic salmon [34] and 64-65 pairs in chum (Fig. 1). It is only the last several pairs in each species that are formed slower.

The fact that dependence of the somite pair number upon the incubation time has the strictly linear character means that formation of somites, one after another, occurs with the one and same temporal interval. This circumstance allows to calculate a given interval as the time of formation of one somite pair τ_s :

$$\tau_s = \frac{\tau_2 - \tau_1}{n_2 - n_1},$$

were τ_1 and τ_2 are the moments when somite pairs n_1 and n_2 respectively started to form. The more is the difference $n_2 - n_1$, the more exact estimation of τ_s can be obtained.

It is necessary to point out the high developmental synchronicity that is observed in embryos of all species and all batches during the period of somi-

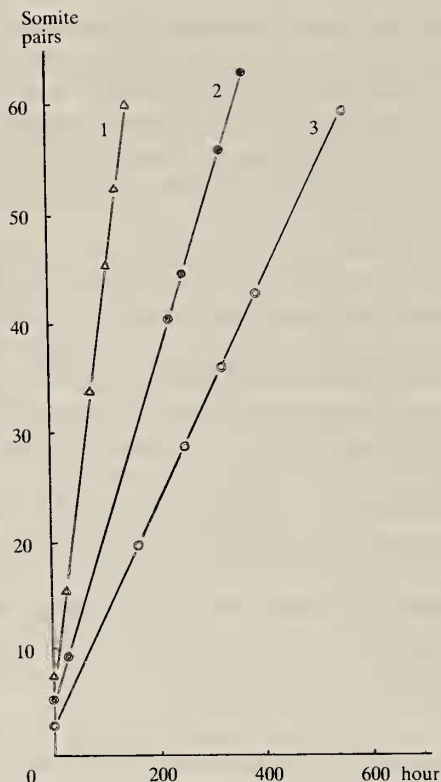


FIG. 1. Increase of the number of somites in chum *Oncorhynchus keta* Walbaum during axial segmentation depending on incubation time at various temperature regimes: 11.3°C (1), 6.0°C (2), 3.7°C (3). Time since the beginning of somitogenesis observations (hours) is marked on the horizontal axis, the number of somite pairs is marked on vertical axis.

togenesis. The synchronicity keeps at the same level at the beginning and at the termination of somitogenesis period. For example, we reveal the data of two plots in Table 1 received from the Atlantic salmon embryos from Kola river population at temperature of 0.4°C. The average value of somite pair number of 12.8 for the first sample had been received from studying of 7 embryos: 6 ones among them had 13 pairs each and one had 12 pairs. After 840 hr (35 days) we have counted somites of 6 embryos from another sample of the same batch. They were 56, 57, 57, 57, 57, and 57 (56.8 in average). We observed the similar level of synchronicity in other instances that can be seen from the meanings of statistic deviation from average numbers of somite pairs (Table 1 and 2).

Thus the somitogenesis at constant conditions appears to be the strictly rhythmic process which is controlled with extraordinary high precision. The data of Table 1 and 2 proves that τ_s is a constant temporal characteristic for all embryos of a given species independently on the time and place of spawn sampling. Deviation of τ_s in parallel samples is usually less than 1% or in some cases 0.1%, that is the absolutely unusual phenomenon for biological processes.

If embryonic development is controlled by any cyclic process, the last one should have the same characteristics in all embryos of a species, but these characteristics can be different in various species, as we see in somitogenesis. For example, in chum and Atlantic salmon at 6.0°C the duration of each cycle of somitogenesis is 368 and 401 min respectively (Table 1 and 2), in pike at 9.9–10.0°C it is approximately twice shorter than in chum (Table 2). It would be said that the embryos of each species have their own time counting off that determine specie-specific "own time" of development.

THE RHYTHMS OF PERIODIC PROCESSES IN TELEOSTS AND AMPHIBIANS AND THEIR INTERCONNECTIONS

If we compare the duration of the cycles of cleavage divisions τ_0 and that of somitogenesis τ_s it is possible to see that at the particular temperature they are similar. We can compare two curves of dependence of these parameters from the incubation temperatures received for Atlantic salmon (Fig. 2). Similar proportions between τ_0 and τ_s are observed in other species. It can be easily traced from the comparison of τ_0 and τ_s values in species having different "own time". As it can be seen from Table 3, species with higher cleavage rate (rainbow trout) also have higher somitogenesis rate and *vice versa* (Atlantic salmon).

In Fig. 3 the duration of the first five cleavage divisions determined cytologically [6] and that of the formation of any five somites at the same temperature (4.8°C) is compared. Here it can be clearly seen that the cleavage rhythm that was lost during the subsequent asynchronous cell divisions seems to reappear during somitogenesis after some

TABLE 1. Determination of time of somite formation (τ_s) in Atlantic salmon (*Salmo salar* L.) embryos from different populations during egg incubation at several constant temperature

Rearing temperature (°C)	Population (river)	Date of onset	Duration of observation (hr)	No. of somites No. of embryos		Somites formed	Time per somite (min)
				onset	end		
0.4	Kola ¹	5/XII	840	$\frac{12.8 \pm 0.15}{7}$	$\frac{56.8 \pm 0.18}{6}$	44.0	1146
	Neva ²	28/XII	818	$\frac{16.0 \pm 0.0}{7}$	$\frac{58.8 \pm 0.17}{9}$	42.8	1147
2.6	Kola	9/XI	580	$\frac{8.7 \pm 0.20}{9}$	$\frac{56.9 \pm 0.18}{8}$	48.2	722
	Neva	6/XII	581	$\frac{8.7 \pm 0.19}{8}$	$\frac{56.6 \pm 0.39}{5}$	47.9	720
6.0	Kola	4/XI	291	$\frac{12.5 \pm 0.34}{6}$	$\frac{56.2 \pm 0.22}{5}$	43.7	400
	Neva	17/XI	335	$\frac{7.0 \pm 0.22}{7}$	$\frac{56.9 \pm 0.21}{8}$	49.9	403
	Salatza ³	14/XI	306	$\frac{8.3 \pm 0.25}{6}$	$\frac{54.3 \pm 0.19}{8}$	46.0	399
9.7	Kola	28/X	164	$\frac{13.0 \pm 0.0}{6}$	$\frac{56.6 \pm 0.19}{8}$	43.6	225
	Neva	11/XI	164	$\frac{9.7 \pm 0.22}{7}$	$\frac{53.2 \pm 0.21}{10}$	43.6	226
	Salatza	9/XI	163	$\frac{8.2 \pm 0.22}{5}$	$\frac{52.0 \pm 0.31}{7}$	43.8	222

¹ Barents sea basin (69° N.L.)² Baltic sea basin (60° N.L.)³ Baltic sea basin (57° N.L.)

hundreds hours.

Incomplete coincidence of τ_0 and τ_s in investigated fish species at several temperatures may be stipulated by different factors. Firstly, the duration of different cleavage cycles, evaluated either by cytokinesis and mitotic index, are not always coincided [37]. Secondly, the activity of inner oscillator is registered only through external processes that can not fully adequately reflect this inner rhythm.

It should be noted that in all fish species studied τ_0 and τ_s give the ratio of 1:1, but this ratio is different in common frog *Rana temporaria*: at 15°C the duration of cleavage cell cycle in frog is 70 min [38] and of the formation of one somite pair is 140 min [39], i.e. the τ_0/τ_s ratio is 1:2. Consequently, for the similar event in reality in one case (fishes) the time equal to τ_0 is spent, in other case

(amphibia) is twice as much. The existence of divisible ratio seems to be caused by the rhythmic processes under discussion submitted to endogenic rhythm generated in embryo with the main period approximately equal to τ_0 . In dependence on complexity and specificity of the process, it manages in one, two and more periods of endogenic rhythm. It can be supposed that the duration of morphogenesis of other organs and that of the period of postulated endogenic rhythm are relating as divisible numbers.

COMPARABLE TRENDS OF CHANGES OF EMBRYONIC RHYTHMS IN INTERSPECIES HYBRIDS

We studied the embryonic rhythms in hybrids between the Pacific salmon species: pink

TABLE 2. Determination of time of somite formation (τ_s) in chum (*Oncorhynchus keta* Walbaum) embryos from different populations and pike (*Esox lucius* L.) embryos from different accessions during egg incubation at several constant temperatures

Rearing temperature (°C)	species	Population (river) or number of accession	Date of onset	Duration of observation (hr)	No. of somites		Somites formed	Time per somite (min)
					No. of embryos			
					onset	end		
6.0	chum	Naiba ¹	11/X-81	336	$\frac{9.0 \pm 0.0}{5}$	$\frac{63.5 \pm 0.27}{8}$	54.5	370
	chum	Zavetinka ²	14/X-86	319	$\frac{12.2 \pm 0.22}{5}$	$\frac{64.1 \pm 0.27}{7}$	51.9	369
	chum	Tym ³	26/X-86	260	$\frac{20.0 \pm 0.22}{7}$	$\frac{62.7 \pm 0.30}{12}$	42.7	364
9.9	chum	Zavetinka	14/X-86	122	$\frac{18.8 \pm 0.25}{6}$	$\frac{55.1 \pm 0.27}{9}$	36.3	201
	chum	Tym	26/X-86	118	$\frac{20.3 \pm 0.31}{7}$	$\frac{55.0 \pm 0.19}{8}$	34.7	204
10.0	pike	1	29/IV	90.5	$\frac{2.2 \pm 0.18}{6}$	$\frac{50.5 \pm 0.22}{6}$	48.3	112
	pike	2	2/V	78.0	$\frac{13.2 \pm 0.19}{8}$	$\frac{56.5 \pm 0.25}{6}$	43.3	108
12.3	pike	1	2/V	54.8	$\frac{14.2 \pm 0.31}{6}$	$\frac{54.8 \pm 0.33}{8}$	40.6	81
	pike	2	2/V	57.4	$\frac{10.2 \pm 0.18}{6}$	$\frac{53.9 \pm 0.12}{9}$	43.7	79

¹ The river on the Western coast of island Sakhalin, entering the Sea of Okhotsk (47.5° N.L.).

² The river on the eastern coast of island Sakhalin, entering the stream of Tartary (47° N.L.).

³ The river in the north of island Sakhalin, entering the Sea of Okhotsk (52° N.L.).

(*Oncorhynchus gorbusha*) and masu or cherry salmon (*O. masu*). It was found out that masu has a higher rate of somitogenesis than pink at all temperatures (Table 4). The comparison of τ_s parent species and hybrids (Table 4) showed that the rate of somitogenesis in hybrids deviated from both parents but was closer to that of male parents.

In 1989–1990 we could not obtain masu females, so we compared τ_s only in pink and in the hybrid where masu was a male parent. At all four temperature regimes τ_s in the hybrid was shorter than in the maternal species.

If cleavage and somitogenesis rhythms have anything in common, the shift in rhythm of one process in a hybrid should be followed by an adequate shift in the other. That was the reason why we studied cleavage rate at one temperature (10.1°C) in masu, pink and their hybrids (Table 5).

In masu cleavage, as well as the somitogenesis,

is completed faster than in pink, that additionally confirm the existence of correlation between the rates of these processes in the same species. The early three cleavage divisions (II–IV) in masu eggs take 83 min less than in pink ones, i.e. τ_0 in masu at 27–28 min shorter than in pink. The τ_0 duration determined as the average time of three cleavage cycles (II–IV) in all variants practically coincided with τ_0 , measured as one cycle duration between cytokinesis of the first and the second divisions.

The measurement of τ_0 in hybrids demonstrates pronounced the shift in the development rate of maternal eggs owing to the influence of father genome: in pink eggs the cleavage rate is enhanced when they were fertilized by the masu males. On the contrary, in masu eggs fertilized by the pink males the cleavage rate became slower (Table 5). Consequently the rhythms of cleavage and somitogenesis in hybrid embryos change by the similar

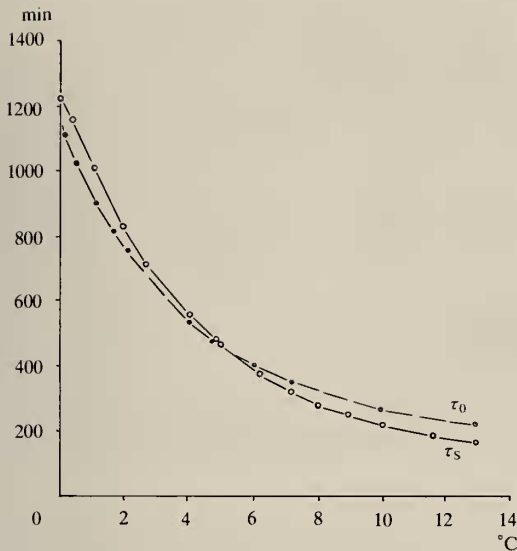


FIG. 2. Duration of cell cycles during cleavage (τ_0) and of the formation of one somite pair during linear somitogenesis (τ_s) in Atlantic salmon embryos depending on the temperature of incubation. Horizontal axis, temperature. Vertical axis, duration.

way: if one process is accelerated, the other process is accelerated too, and *vice versa*.

It should be noticed that the inheritance of the cleavage rate in hybrids of considered variant is evidently influenced by the male parent. It breaks up the old presentation that the cleavage rate is dependent on the egg cytoplasm only (for review

see [13]). Thus the evident similarity in the rhythms of cleavage and somitogenesis, taking place at the different periods of embryogenesis, definitely proves the existence of common biological time in embryos for these periods, and perhaps for other periods of embryo development as well.

AGE-RELEVANT CHANGES IN COMPETENCE

Competence is defined as the ability of embryo tissues to respond to an inductive signal that activates particular developmental programmes [41–42]. It has been found that the competence of the embryo tissues changes with time, following a strict programme.

Holtfreter [43] observed the changes in the ability of isolated ectoderm of the early gastrula cultivated for different periods in a salt medium to form different rudiments. It is important that these changes occur in a stepwise and autonomously. This was confirmed by recent studies.

It was shown that mesodermal competence is lost not only in isolated ectoderm, but in dissociated division-arrested cells on the same time schedule as in whole embryos [22]. Loss of neural competence, and the gain and loss lens competence can all occur in isolated ectoderm. The major conclusion is that these changes in competence take place as a result of intrinsic and autonomous processes, occurring within the cells of

TABLE 3. Comparison of time (min) of cell cleavage (τ_0) and somite formation (τ_s) at identical temperatures in embryos of three salmon species¹ (Salmonidae)

Species	Atlantic salmon		Chum		Rainbow ² trout	
Temperature 0°C	τ_0	τ_s	τ_0	τ_s	τ_0	τ_s
3.0	657	670	570	654	514	527
4.0	558	555	480	538	433	431
5.0	479	470	408	452	367	357
6.0	415	397	350	374	314	300
7.0	364	328	303	317	270	254
8.0	321	284	265	270	234	219
9.0			234	232		
$\pm \delta$	16.7	3.9	7.3	16.9	20.9	4.6

¹ The value of τ_0 and τ_s are obtained by nonlinear regression [35] from the own data.

² The value of τ_0 for rainbow trout embryos is calculated from the data of Ignatieva [36].

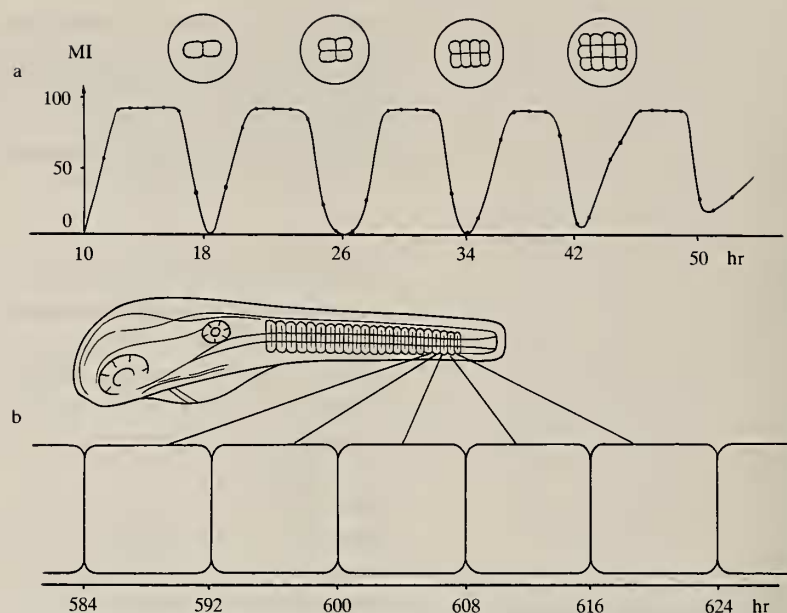


FIG. 3. Comparison of the duration of cell cycles of the first five cleavage divisions and of the formation of five successive somites (from 19th to 23rd) during linear somitogenesis in Atlantic salmon embryos at constant temperature. Horizontal axis (a, b), time of incubation at 4.8°C from the moment of fertilization. Vertical axis, (a) changes of mitotic index (MI) (data of Gorodilov and Lilp, 1978).

TABLE 4. Time of somite formation (τ_s) in pink and masu embryos (Pacific salmon from genera *Oncorhynchus*) and their hybrids at identical incubation temperature

Year of observation	Temperature of incubation (°C, ± 0.05)	Value of τ_s (min)			
		pink	masu	♀ pink × masu ♂	♀ masu × pink ♂
1987	12.5	148	137	—	149
1988	6.6	327	310	314	—
1989	4.6	481	—	458	—
1989	6.4	344	—	333	—
1989	12.4	150	—	140	—
1990	5.9	379	—	368	—

ectoderm [23]. Besides, in some experiments it was observed that inducing and reacting abilities of developing tissues and cells gain or loss abruptly [23, 44–45]. As Gurdon [41] has emphasized, in the analysis of induction it is important to be able to analyse a rapid response that can be observed in single cells. It may be presumed that the changes in development occur in discrete steps the duration of which is equal or proportional to τ_0 and τ_s .

THE ROLE OF DEVELOPMENTAL CLOCK IN THE REGULATION OF EMBRYOGENESIS

The evidence of rhythmic processes occurring at various stages of embryogenesis definitely indicates the presence of a time control mechanism in the embryos. The time count probably begins at the moment of fertilization and might continue

TABLE 5. Determination of earlier cleavages duration and τ_0 values in embryos of pink, masu and their hybrids at temperature 10.1°C

Species of embryos	Number of the embryos studied	Time (min) ¹ from insemination up to beginning in 50% embryos cytokinesis of			Duration of one cell cycle τ_0 (min)	
		first cleavage τ_I	second cleavage τ_{II}	forth cleavage τ_{IV}	$\tau_{II} - \tau_I$	$\frac{\tau_{IV} - \tau_I}{3}$
pink	113	533 ± 2.5	765 ± 2.8	1240 ± 3.5	232 ± 1.8	236 ± 1.2
masu	125	575 ± 2.7	780 ± 1.9	1199 ± 3.6	205 ± 1.6	208 ± 1.3
♀ pink × masu ♂	109	585 ± 4.9	802 ± 3.8	1245 ± 2.7	217 ± 1.7	220 ± 1.4
♀ masu × pink ♂	116	573 ± 4.0	790 ± 3.5	1250 ± 3.3	217 ± 2.7	226 ± 1.6

¹ The deviations of the data was estimated by the Berehns formula [40]

until the end of embryogenesis. We consider this mechanism to be responsible for the general schedule of development. It keeps all processes within particular time limits. This system can be imagined as a kind of metronom with oscillation period equal or proportional with ration 1:1, 1:2, 1:3, etc. to the duration of different processes, steps and stages of differentiation and morphogenesis, oscillations of which influence all embryonic cells.

We suggest that in the egg the signals which are received by all embryonic cells are generated periodically and serve to switch on/off expression of genes (or clusters of genes), programmes and subprogrammes of differentiation and morphogenesis in various types of cells and parts of the embryo depending on the serial number of a particular signal. This endogenic pacemaker with regular time intervals triggers the development of cells and multicellular structures in different pre-set directions.

We tried to show this scheme of development in Fig. 4. The time schedule of the development beginning from fertilization with (τ_0 or τ_s if these intervals are equal) as a time unit is shown on the left. The stages of development of some embryonic structures, parts, organs which are started or finished simultaneously, i.e. at one and the same controlling signal of a clock are indicated at a horizontal line. This scheme is based on our data on embryo development in salmon fishes. For example [3, 33] during the first τ intervals the cleavage divisions occur, after 12 τ from the start

of development blastula with enveloping and deep cell layers and periblast is formed, after 56 τ the axial organs are formed and somitogenesis begins, etc. By 350 τ embryogenesis is completed in general and morphologic development is mostly over.

According to this scheme the developmental programmes for various differentiations are released in time by the embryo clock so that each of them is switched on according to the serial number of a clock signal. Under such condition, various programmes can be completed independently. This would explain the fact that some cyclic processes in embryonic cells continue when cytokinesis or nuclear divisions are suppressed [26]. This can be also the cause when isolated cells and tissues of cartilage [27] or of amphibian ectoderm [22, 23] kept their developmental schedule by the same way as if it had been happened in the intact embryos. At the same time some processes are, of course, interdependent due to induction, hormonal and other interactions in the embryo.

What happens if the formation of a structure is not completed by the time of a new signal? Probably, in the case of vitally important organs (heart, brain, kidneys) it is lethal to the organism, but in the case of less important organs the resulting abnormality can be retained and at the new time signal the organ enters the next stage of development. For example, when deformations were produced in somites by heat shock, further development of somites continued normally and with

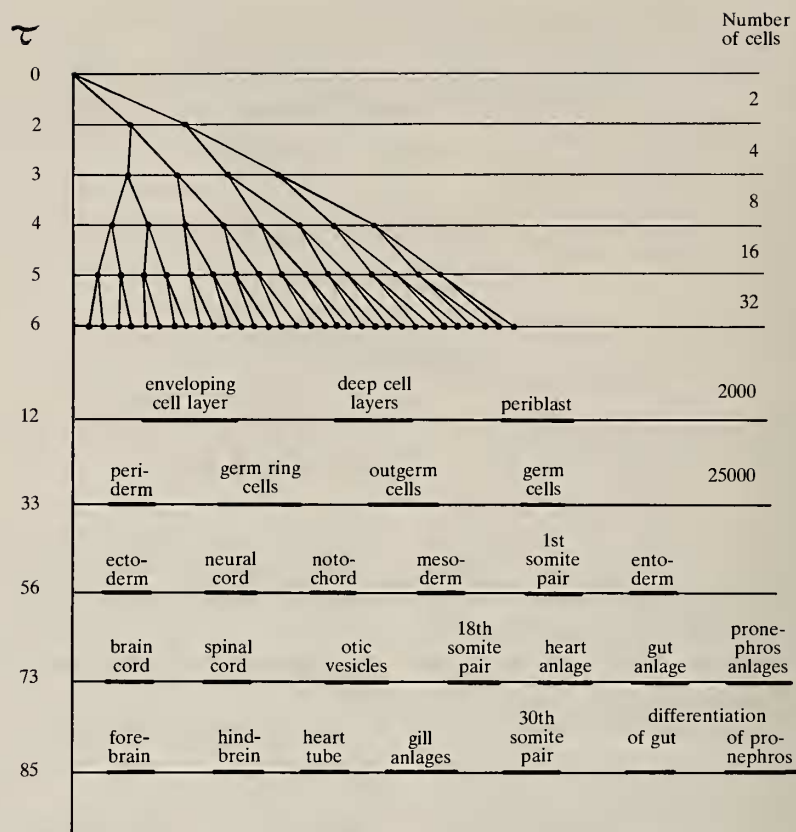


FIG. 4. The scheme of salmon embryos development using τ as a time unit (see explanation in the text).

normal rate [46, 47].

Thus, the time factor in animal embryogenesis is not only one of the coordinates of the process of organism formation, but it is a *primary* factor actively monitoring the development with the help of periodically generated endogenic rhythms. The latter provides the mechanism which measures the embryo inner time and thus defines the order of functioning of embryo cells and multicellular structures.

In contrast with the formed adult organisms in which timing is based on periodic astronomic processes and hence is common in all species, we think the time in embryos is counted by a species-specific temperature-dependent internal periodic process (of unknown nature). After completion of embryogenesis the individuals gradually acquire outer universal rhythms which direct their living.

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